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**Originals**

# A Rediscovery of Androdioecy and Pollen Formation in the Hermaphrodite Flower in Nagaimo (*Dioscorea opposita* Thunb.)

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Seeds of the wild type of Nagaimo, *Dioscorea opposita*, were introduced from the People's Republic of China in 1995. The obtained seedlings contained both male and female plants (Kanazawa et al. 2002), and produced abundant seeds under natural conditions in Morioka, northeast Japan. These seeds germinated and seedlings grew. The male plant produced flowers bearing normal stamens and the degenerated pistil. The female plant, we initially thought so because the genus *Dioscorea* has been regarded to be a dioecious plant group with unisexual flowers in each plant, produced hermaphrodite flowers. Thus this Nagaimo turned out to be androdioecious. Embryo sac formation is of the *Polygonum* type. The filament terminal of the stamens in the hermaphrodite flower, when observed from above, was considerably smaller than that of the male flower. The pollen morphology showed two types in the hermaphrodite flower; one was normal globular shape as the pollen grains of male flowers, and the other was deformed shape of empty hemisphere like a degassed rubber ball.

**Key words**: Abnormal pollen grains, androdioecism, *Dioscorea opposita*, gynodioecism, hermaphrodite flower.

Chinese yam, *Dioscorea opposita* Thunb., belongs to the section Enantiophyllum of the genus *Dioscorea* (Burkill 1960). This section includes many edible species. Chinese yam has been cultivated for a long time in Japan, Korean Peninsula as well as China, the probable home of this species.

Some researchers considered the genus *Dioscorea* to be unisexual (Ayensu 1972, Caddick et al. 2002), but others did not clearly deny the existence of hermaphrodite flowers in the genus (e.g., Burkill 1960). And it has been believed that almost all the Nagaimo plants, one of the three major cultivars of *D. opposita*, are male in Japan. But, the existence of hermaphrodite strain has been reported earlier by Mizuno (1955), and the existence or production of female strain has also been reported by Yakuwa et al. (1981) independently, though these exceptions do not seem to be familiar.

Finding out the hermaphrodite flowers, Mizuno (1955) described that "only a few to some flowers fertilize in an inflorescence. There are two strains; one fertilizes and produces seeds to some extent, another produces male flowers." The two strains in Mizuno's description would mean that Nagaimo is an androdioecious plant. Nagaimo reported by Yakuwa et al. (1981) very rarely produced

fully matured seeds in the natural condition of Hakkaido, north Japan.

Nagaimo which we used in the present study contained both male and 'female' strains and, differing from both of the above described Japanese strains 'female' plants produced abundant fertile seeds (Kanazawa et al. 2002). Through the continuous observation of the cultivating Nagaimo, we noticed that many flowers of both male and 'female' plants often kept closed throughout a growth season and that the swelling of ovary, nevertheless, seemed to begin before anthesis, though usually with small aperture, of male flower around the 'female' plants. In this report we mainly described 'female' flower morphology as a basic study of the mechanism of seed formation.

## Materials and Methods

**Seeds from China:** The plant was identified to be a wild type of Nagaimo, *Dioscorea opposita* Thunb. with long underground tuber, by one of the authors (Ding), and the seeds were collected by Terui and Ding at natural habitats in the Mount Wudang, Hubei Province, the People's Republic of China in the late October of 1995. The seeds were induced to germination in 1995–1996 in Japan and the seedlings have been subcultured since then using tubers (Kanazawa et al. 2002). Voucher specimens have been deposited in the Herbarium of Tohoku University (TUS).

**Observation of flowers with light microscope:** Flowers of various developmental stages were harvested and fixed with Carnoy solution (acetic acid: ethanol: chloroform = 1 : 6 : 3 v/v/v), dehydrated with an t-Butanol series, and embedded in paraffin. Sections of 8 μm thick were double stained with safranin and fast green.

**Observation with scanning electron microscope (SEM):** Fresh materials were fixed with glutaraldehyde (2 % in 0.1 M phosphate buffer, pH 7.3), in a refrigerator for 2 hrs. Then they were transferred to 1 % osmium tetroxide solution for postfixation for more than 2 hrs. After dehydration through an ethanol series, isoamyl acetate was used for infiltration. The materials were then dried at the critical point (Critical point drier, Hitachi, HPC-1) and coated with gold vapor in an ion coater (Eiko Engineering, IB-3). The observations were made with a SEM (Hitachi, S-2300).

## Results

Both male and 'female' plants of Nagaimo derived from China produced many flowers on many spikes (Fig. 1). Distances between flowers elongated in 'female' flowers (Fig. 2). Stems twined around the prop and then slid down for its weight when abundant seeds were produced in the capsules (Fig. 1C).

In the flowers of both sexes, perianth does not open so wide (Figs. 3A, 3B) differing from that of the section Stenophora, for example, *D. tokoro*, the most popular wild *Dioscorea* species in Japan. After removing the perianths, stamens are observed in the male flower (see Fig. 3), and degenerated pistil is observed after removing the stamens. In the 'female' flower, which is epigynous, well developed stamens are observed as well as a pistil. Stamens in the male flower have relatively large-swelled filament terminal and smaller anthers compared to those in the 'female' flower (compare Fig. 3C with Fig. 3D). Even in the unopened 'female' flower it was often observed that abundant masses of sticky pollen grains adhered to the stigma after removing the perianth (Figs. 3E, 3F).

Anthers of the inner and outer whorls tightly surrounds the pistil with trilocular ovary (Fig. 4A), and have pollen grains in the anthers (Figs. 4B, 5G). Thus the 'female' flower is called 'hermaphrodite' flower hereafter. Serial sections of the hermaphrodite flower showed various stages of microsporogenesis (Fig. 5).

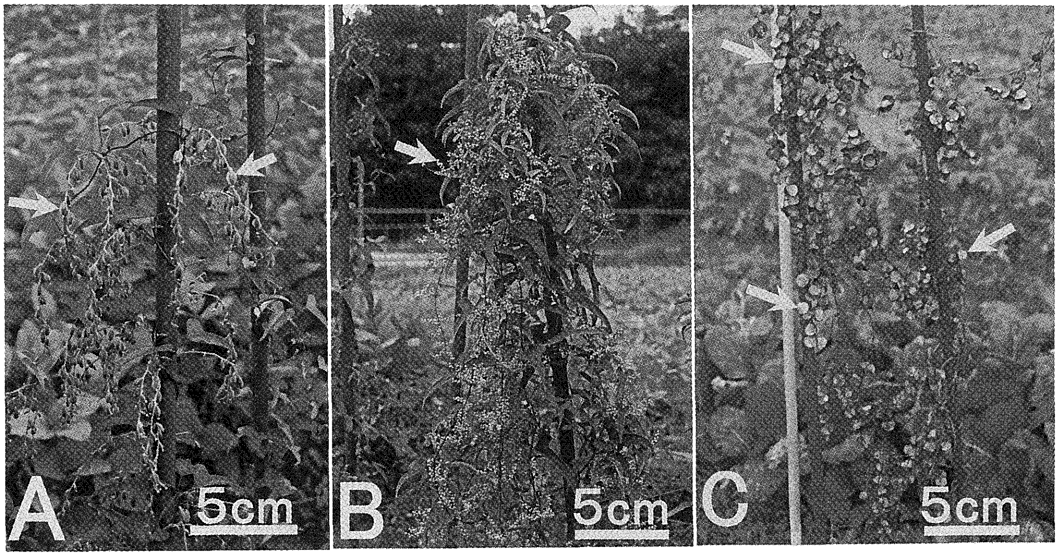
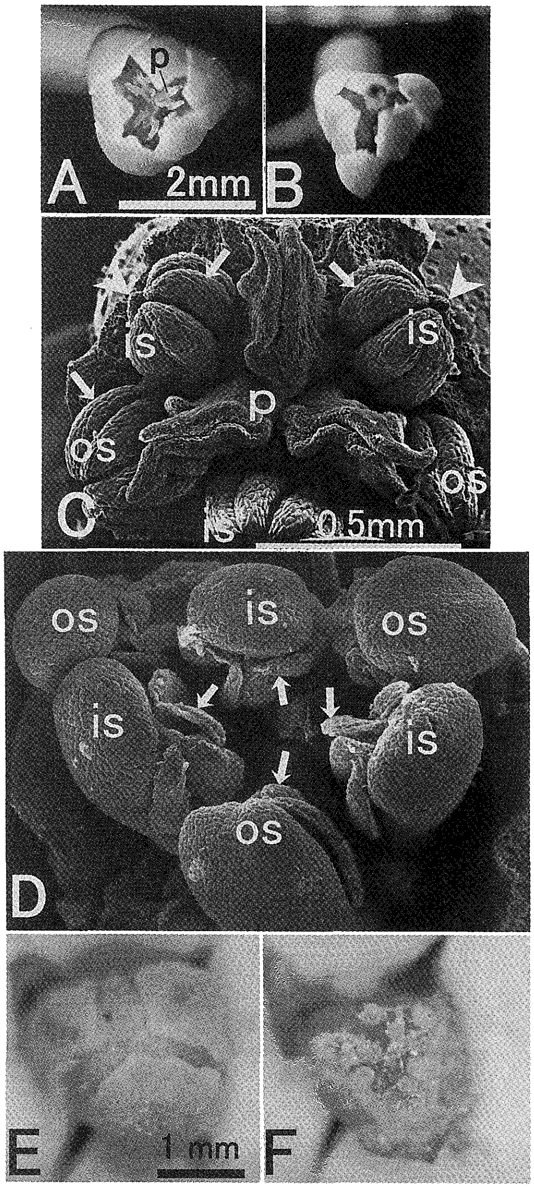



Fig. 1. Nagaimo (*Dioscorea opposita*) plants. A. Developing fruits (arrows) seen on 'female' spikes in July. B. Male flowers (arrow) seen on male plants. C. Mature fruits (capsules, arrows) seen on a 'female'plant in autumn.


Fig. 2. Flowers of Nagaimo (*Dioscorea opposita*). A. 'Female' spike with epigynous flowers (arrow). Arrow head indicates ovary. B. Male spikes at a node with sessile flowers (arrow).

Almost all the pollen grains produced in male anthers were globose (Fig. 6C), however, the hermaphrodite anther produced two types; one was globose like the male pollen grains, and the other was a empty hemisphere like a completely or half degassed rubber ball (Figs. 5G, 6A, 6B). The ratio of the numbers of globose pollen grains and hemisphere ones changed year by year, perhaps depending on the weather conditions.

Embryo sac formation was *Polygonum* type as in *D. nipponica* and *D. tokoro* (Takeuchi and Kimura 1968). Seeds, produced in the hermaphrodite flowers under the natural condition in the present experiment, germinated at the rate of near 100 % at the optimal germination temperature (about 11 ℃) except for the very small number of seeds with very thin and probably empty endosperm (data not shown).




## Discussion

Almost all the plants of the two cultivars, Ichoimo and Tsukuneimo, are female, while those of the cultivar Nagaimo are male (Yakuwa et al. 1981) in *Dioscorea opposita*. Nagaimo has been reported, though very rare, to have both male and hermaphrodite strains in Japan, and the latter produced some seeds in Osaka Prefecture., southwestern Japan (Mizuno 1955). Nagaimo produced more seeds in Yamagata. (northern Japan) than in Osaka (Aoba 1968). We confirmed the presence of hermaphrodite flower in Nagaimo derived from China and the hermaphrodite plants produced abundant seeds. These facts reveals that Nagaimo is androdioecious.

Further, both Ichoimo and Tsukuneimo

Fig. 3. Male and 'female' flowers of Nagaimo (*Dioscorea opposita*). A. A 'female' flower. Pistil (p) is seen from above through widely opened perianths. B. An opened male flower. C. A scanning electron micrograph of a young 'female' flower after removing perianths. Arrows indicate anthers and arrow heads indicate filament terminals. D. A scanning electron micrograph of a male flower after removing perianths. The letters 'is' and 'os' show filament terminals and arrows indicate some of the anthers. E. An unopened 'female' flower. F. Inside view of the unopened flower (Fig. E) after removing the perianths. Pollen grains adhered to the stigmata are seen. Abbreviations: is, stamen on the inner whorl; os, stamen on the outer whorl; p, pistil.

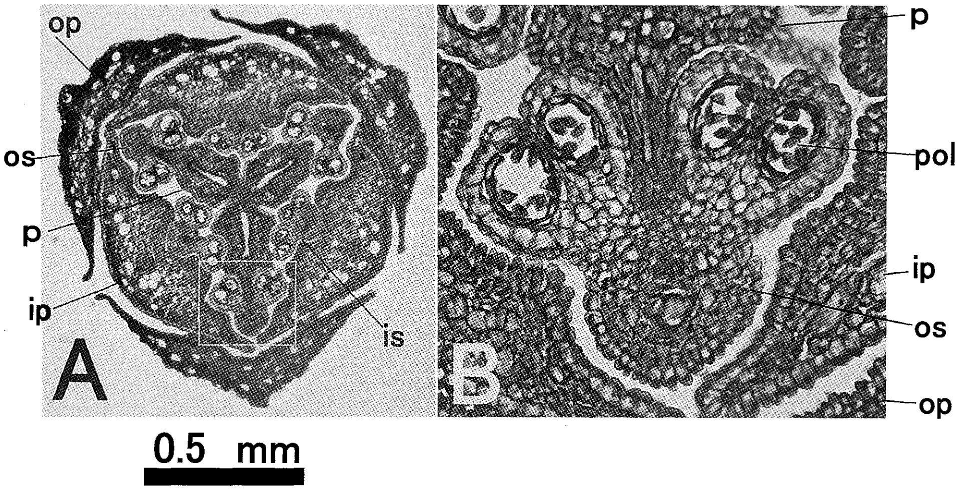



Fig. 4. Cross section of a ‘female’ flower of Nagaimo (*Dioscorea opposita*). A. A whole flower above the ovary. B. An enlargement of the framed stamens of Fig. A. Abbreviations: ip, inner perianth; op, outer perianth; is, stamen on the inner whorl; os, stamen on the outer whorl; p, pistil; pol, pollen.

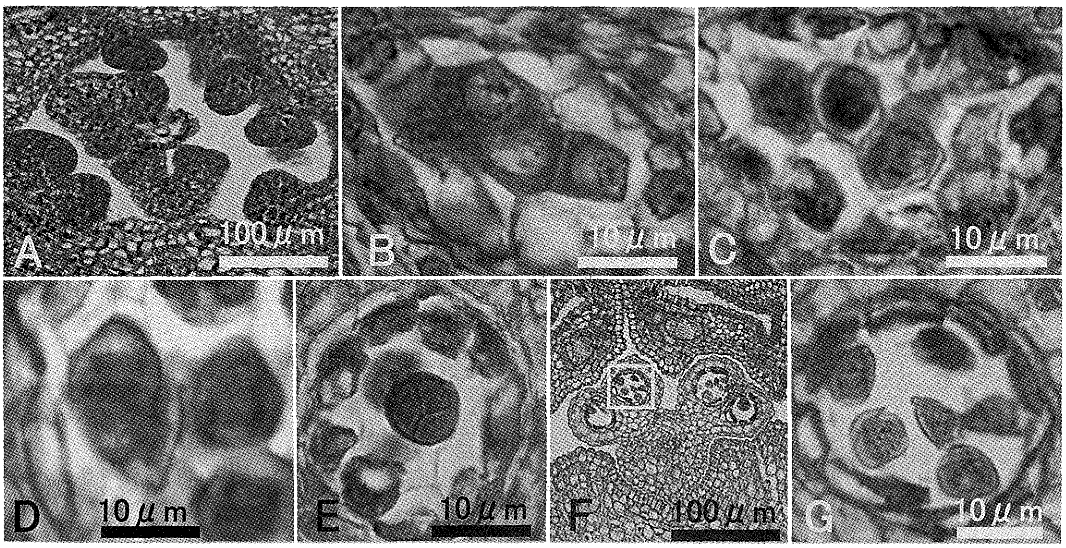



Fig. 5. Pollen formation in the hermaphrodite stamen of Nagaimo (*Dioscorea opposita*). A. Archesporial cells. B. Pollen mother cells. C. Anaphase II. D. Telophase II. E. Pollen tetrad. F. Pollen grains in the anthers. G. An enlargement of the framed part of Fig. F.

have hermaphrodite flowers irrespective of general belief in the existence of female strain alone (Mizuno 1953), but there have been no report showing the presence of male plants. The hermaphrodite flowers produced fertile pollen grains which lead to the formation of seeds with germination ability (Matsuo and Mizuno 1954). These information may suggest that Tsukuneimo and Ichoimo are gynodioecious.

Unisexual flower is considered to have evolved from hermaphrodite flower (Kawano 1974). The genus *Dioscorea* has unisexual flowers in general. But Nagaimo has three strains: male strain, female strain (Yakuwa et al. 1981), and hermaphrodite strain. Therefore, *D. opposita* could be an exceptional species in the genus, which is on the way of evolution from hermaphrodite plant to unisexual one, and the number of plants with hermaphrodite flowers might have been decreasing. This characteristics of this species would be useful as a material for the study of mechanism of sex differentiation and sex determination or their history in the evolution.

The rare production of seeds in the female or hermaphrodite Nagaimo, as described above, differs from the abundant production in the present material. We cannot explain how occurred the difference, however, the chromosome number may give a hint to this problem. The chromosome number has been reported to be 2n = ca. 140 for *D. opposita* both in Japan and China (Nakajima 1933, Araki et al., 1983, Chin et al. 1985), but the present material has the chromosome number of 2n = 80 at the root tip cells of the seedlings (unpublished data). The chromosome numbers of Old-World species of the genus *Dioscorea* has been known to be multiples of x = 10 (Martin and Ortiz 1963). And it is generally known that growth and/or reproduction of polyploid plants is more or less different from the diploid plant or from others with different ploidy.

Caddick et al. (2000), in a series of investigation on the taxonomy of *Dioscorea* and related species, showed scanning electron micrographs of flowers of some *Dioscorea* species. They regarded the seeming stamens in the 'female' flower as staminodes and described that the flowers were unisexual. But these staminodes resemble the stamens of the present material. Staminode has been considered generally to be 'sterile or abortive stamen, or its homologue, without an anther (Jackson 1971)'. The definitions of the term 'stamen' and 'hermaphrodite flower' have not necessarily been clear, so we use the term 'hermaphrodite flower' to indicate flowers which have both stigma and stamen(s) with pollen grains in the anthers irrespective of the reproductive ability.

Sadik and Okereke (1975) found complete flowers on the spike of staminate flower in *D. rotundata* (section Enantiophyllum), an African edible plant. He described "All attempts to germinate pollen grains, collected from flowers of several developmental stages,..., have not been successful, except in few cases." Several causes have been given for the production of abnormal pollen grains or lack of germination ability; for example, pollen formation seemed to have genetic basis in the genus *Oxalis* (Dreyer and van Wyk 1998), it was affected by day-length in *Salsola komarovii* (Takeno et al. 1996), but it was affected by temperature as reported in some varieties of rice plants (Satake 1976). The formation of empty pollen in Nagaimo in the present study (Fig. 6) seemed to depend on the smallness of accumulated temperature and/or accumulated day-length during the growing season. The fruits as well as seeds was considerably few and no or few fruits were observed on each plant in the years with cold summer, as if it were different strains from that shown in Figs. 1A or 3. To clarify the relation between weather condition and the pollen formation, observations are in progress using fixed flowers collected

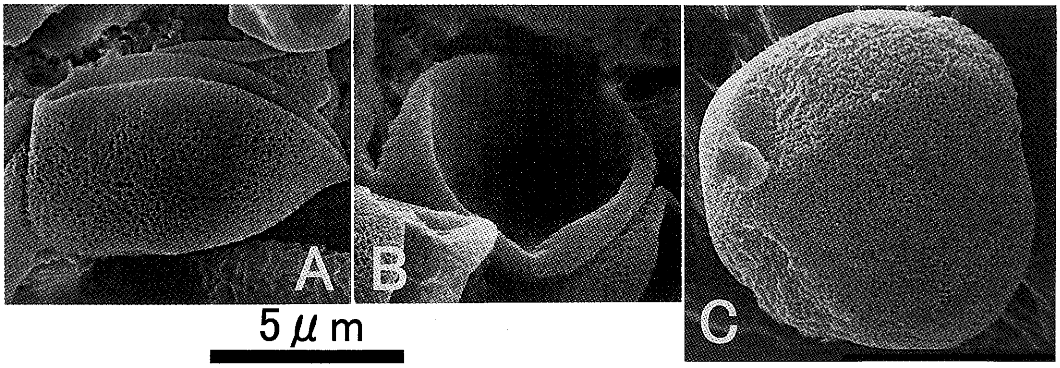



Fig. 6. Pollen grains in hermaphrodite and male flowers of Nagaimo (*Dioscorea opposita*). A, B. Abnormal pollen grains often found in the hermaphrodite flowers. C. A normal pollen grain found in the male anthers. Hermaphrodite flowers also have similar pollen grains.

in such years. In the plants which are on the way of evolution from hermaphrodite flower to unisexual (female or male) one, genetic instabilities, if exists, could also be one of the causes of the production of empty pollen grains.

On the difference in the sizes of filament terminals between male and hermaphrodite flowers observed in the present study (Fig. 3), we have found no related reports except for Caddick et al. (2000) in which filaments in the male flowers seems not so large as 'female' ones. Implications of this difference remains obscure at present.

Whether a hermaphrodite flower alone, with no participation of male flower, produces seeds in our material has not been certain yet.

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## Endnote

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ナガイモの野生株の種子を1995年に中華人民共和国から導入した．得られた実生には雌雄両株があり，盛岡の自然条件下で多数の種子を生じた．種子は発芽し，成長した．ヤマノイモ属は雌雄異株で，それぞれの株に単性花を生ずると考えられてきた．しかし，本種では雄株の花には雄しべのみが生じ，雌しべは痕跡だけであるが，雌株は雌しべに加えて，花粉を生ずる雄しべを備えた両全花を生じた．したがって，このナガイモは雄性両全性異株であることが分かった．胚嚢形成は*Polygonum* type である．雄花の雄しべの花糸は上から見ると，両全花のものよりかなり大きい．さらに，両全花の花粉粒には二つのタイプが認められたが，一方はおしべの花粉と同様な球形，他方はゴムのボールから完全に空気を抜いたような空の半球形の異常型であった．

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